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28 February 1984; accepted 1 August 1984

Chromosomal Location of Human T-Cell Receptor Gene $T_i\beta$

Abstract. *A complementary DNA probe corresponding to the β-chain gene of T_i, the human T lymphocyte receptor, has been molecularly cloned. The chromosomal origin of the $T_i\beta$ gene was determined with the complementary DNA by screening a series of 12 cell hybrid (mouse × human) DNA's containing overlapping subsets of human chromosomes. DNA hybridization (Southern) experiments showed that the human $T_i\beta$ gene resides on chromosome 7 and is thus not linked to the immunoglobulin loci or to the major histocompatibility locus in humans.*

Studies of both human and murine systems have shown that the receptor for antigen and major histocompatibility complex on T lymphocytes belongs to a novel class of 80- to 90-kilodalton (kD) disulfide-linked heterodimer that expresses clonotypic epitopes (*1–5*). In humans, these molecules—termed T_i—are each comprised of one 49- to 51-kD α subunit and one 43-kD β subunit that are, in turn, noncovalently associated with the invariant 20- and 25-kD T3 molecules (*6*). Recently, amino-terminal amino acid sequencing and molecular cloning techniques have identified the $T_i\beta$ gene and shown that it has a distant but definite homology with immunoglobulin light chains (*3, 6–9*). The availability of complementary DNA (cDNA) probes for the $T_i\beta$ genomic sequence (*7*) makes it possible to ascertain its chromosomal position.

We constructed a genetic mapping panel of hybrids of mouse × human somatic cells that contain overlapping subsets of human chromosomes on a rodent genetic background. In effect, the panel served to isolate small groups of human chromosomes that could be screened for the presence of the $T_i\beta$ gene by nucleic acid homology with the corresponding cDNA probe. The distribution of human chromosomes across the panel was determined by isoenzyme and karyotype analysis (*10*) and in certain cases by human DNA probes whose map location has been established. The distribution of $T_i\beta$ genomic sequences across the panel was independently determined by molecular hybridization of cell hybrid DNA with an isotopically labeled $T_i\beta$ cDNA probe (*7, 11*). Comparison of the two sets of data enabled assignment of any hybridization band to a specific human chromosome. Further, segregation of various $T_i\beta$-specific bands with respect to each other gave an indication of genetic linkage among bands. This approach therefore allowed chromosomal assignment of the structural gene as well as unlinked but related human $T_i\beta$-like DNA sequences. Samples of human, mouse, or mouse × human cell hybrid genomic DNA's (20 μg each) were cleaved with restriction endonuclease Eco RI, separated by agarose gel electrophoresis, transferred to nitrocellulose, and probed with the pβREX plasmid for the $T_i\beta$ gene (*7*) (Fig. 1). Genomic mouse A9 DNA cleaved with Eco RI (lanes c and k) showed a single intense 2.3-kilobase (kb) Eco RI band. In addition, two very large, less intense bands representing mouse DNA were observed, both greater than 9.5-kb. Under these conditions, their sizes could not be accurately determined. Human HeLa DNA cleaved with Eco RI (lanes d and l) contained four intensely hybridizing bands with apparent sizes of 2.0, 5.2, and 7.0 kb and a very large band in excess of 9.5 kb. These results indicate that somatic cell hybrid DNA's cleaved with Eco RI and probed with isotopically labeled pβREX should each contain mouse DNA–specific bands corresponding to the mouse A9 DNA genetic background. In addition, those hybrid cell DNA's that contain the chromosome bearing the human $T_i\beta$ gene should contain human DNA–specific Eco RI bands.

Twelve hybrid (mouse × human) somatic cell DNA's were screened by Southern blotting (*12*) for the presence of human $T_i\beta$ DNA sequences. As predicted, each cell hybrid DNA (Fig. 1, lanes e to j and m to r) contained the 2.3-kb mouse DNA–specific $T_i\beta$ band. Two hybrids, FRY4.A+SEG and 53-87(3)cl.10 (Fig. 1, lanes h and j, respectively), showed all three human DNA–specific $T_i\beta$ bands between 2.0 and 7.0 kb. The large band representing human $T_i\beta$ DNA also appeared to be present in these two hybrid DNA's; however, mouse DNA–specific bands migrating together with this band obscured the analysis. A third somatic cell hybrid DNA, BDA 14b25

Table 1. Chromosomal content of somatic cell hybrid cell lines assayed by cytogenetic and isoenzyme analysis (*10, 15, 22*). Chromosomes listed include translocation chromosomes present.

Hybrid cell DNA	Human chromosomes present	Blot hybridization result with pβREX
BDA 10a3	2,3,5,6,8,9,10,11,13,14,16,17,20,22,X	−
BDA 10a4aF9-1	2,4,5,6,8,12,14,X	−
BDA 17b17	1,3,4,5,9,12,14,15,18,19,20,21,22,X	−
FRY 4.A+SEG	1,2,3,4,6,7,8,10,11,12,14,15,18,21,22,X	+
BDA 14b25	1,2,3,4,7,12,14,15,X	+*
53-87(3)cl.10	7	+
AHA 16e	10,11,12,13,14,17,18,19,20,21,X	−
AHA 3d2-2	1,4,15	−
AHA 3d2-3	3,4,8,11,12,18,19	−
AHA 16e-3	1,3,4,10,11,12,13,19,21,X	−
AHA 16e-6	1,2,3,4,10,11,13,14,16,19,20,X	−
41pT2A	1,3,4,8,10,12,14,15,16,18,19,21,X	−

*Weak positive hybridization.

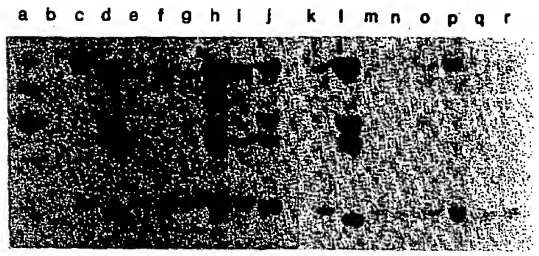

Fig. 1. Human (lanes d and l), mouse (lanes c and k), and somatic cell hybrid (lanes e to j and m to r) genomic DNA's cleaved with Eco RI and probed with pβREX plasmid (*11*), a pBR322 construct containing a full-length cDNA of about 1.3 kb encoding the $T_i\beta$ subunit of the human T-cell receptor from the human T-lineage tumor REX. Somatic cell hybrids were characterized by isoenzyme and karyotype analysis (*10*), and genomic DNA's were isolated (*15*) and blotted (Southern) by established methods (*12, 22*). The probe was hybridized (*22*) to filters that were subsequently washed at high stringency (*22*). End-labeled (*23*) marker DNA's were restriction fragments of Hae III–cleaved φX174 and Hind III–cleaved bacteriophage lambda. Hybrid cell DNA's were BDA 10a3 (lane e), BDA 104aF9-1 (lane f), BDA 17b17 (lane g), FRY4.A+SEG (lane h), BDA 14b25 (lane i), 53-87(3)cl.10 (lane j), AHA 16e (lane m), AHA 3d2-2 (lane n), AHA 3d2-3 (lane o), AHA 16e3 (lane p), AHA 16e6 (lane q), and 41pT2A (lane r). Marker DNA's in lane a migrate at the sizes given to the left (in kilobases).

(Fig. 1, lane i), showed a low-intensity positive signal for the human $T_i\beta$ gene. The remaining nine somatic cell hybrid DNA's contained only mouse DNA–specific $T_i\beta$ bands. Lane p contained a background haze and was clearly negative for the signal for human DNA upon overexposure of the blot (data not shown).

Only human chromosome 7 corresponded to the distribution of human $T_i\beta$ gene sequences across the panel (Fig. 1 and Table 1). A concordance analysis of these data (Table 2) showed complete concordance between human chromosome 7 and the $T_i\beta$ gene. Each human chromosome other than human chromosome 7 was ruled out as the site of human $T_i\beta$ gene by four or more cell hybrid DNA's. Most convincing, however, was the strong positive signal in lane j (Fig. 1). This hybrid, 53-87(3)cl.10 (*13*), contained only human chromosome 7 on a mouse genetic background.

As confirmation of the assignment, the filters used for Fig. 1 were stripped and hybridized again with the λ he-B probe that is a 15-kb Eco RI insert of the human c-*erb* B gene into λ Charon 4A (*14*). This gene has been mapped to human chromosome 7 (*14*). In our control experiment (data not shown), Eco RI bands specific for human c-*erb* B with lengths of about 7.0 and 2.0 kb appeared in lanes c, h, i, j, and l, confirming the distribution of human chromosome 7 across the mapping panel that had been previously determined by isozyme and karyotype analysis (*10, 15*). We are thus confident that human chromosome 7 contains the $T_i\beta$ gene.

These results also indicate that three of the four human DNA–specific bands represent human chromosome 7 sequences. A faint 9.0-kb Eco RI human DNA–specific band (Fig. 1, lanes d and l) could not be assigned by these data. This band probably represents a distantly related human V-β gene; however, further work is required to establish its origin.

Our finding that human chromosome 7 contains the $T_i\beta$ gene indicates that this locus segregates independent of the immunoglobulin κ light-chain gene on human chromosome 2 (*16*), the λ light-chain locus on human chromosome 22 (*17*), or the heavy-chain complex on human chromosome 14 (*18*). The $T_i\beta$ gene is also not genetically linked to the major histocompatibility locus on human chromosome 6 (*19*). Several genes have been mapped to human chromosome 7, including the epidermal growth factor–c-*erb* B oncogene (*14*) and the SV40 integration site (*13*), but the $T_i\beta$ gene represents the first T- or B-cell product to be associated with this chromosome. In future studies, the map location of the human $T_i\beta$ gene merits consideration among cytogeneticists and molecular biologists studying specific chromosome 7 changes (*20, 21*) in human leukemias.

Table 2. Concordance analysis of T-cell receptor β-chain gene ($T_i\beta$) assigned to human chromosome 7 by molecular hybridization of cDNA probe pβREX to 12 hybrid (mouse × human) somatic cell DNA's cleaved with Eco RI.

Human chromosome	Hybrid cell DNA's (number)				Discordant DNA's (total)
	Concordant*		Discordant		
	+/+	−/−	+/−	−/+	
1	2	4	1	5	6
2	2	6	1	3	4
3	2	3	1	6	7
4	2	2	1	7	8
5	0	6	3	3	6
6	1	7	2	2	4
7	3	9	0	0	0
8	1	5	2	4	6
9	0	7	3	2	5
10	1	4	2	5	7
11	1	4	2	5	7
12	2	3	1	6	7
13	0	5	3	4	7
14	2	3	1	6	7
15	2	6	1	3	4
16	0	6	3	3	6
17	0	6	3	3	6
18	1	5	2	4	6
19	0	3	3	6	9
20	0	5	3	4	7
21	1	5	2	4	6
22	1	7	2	2	4
X	2	2	1	7	8

*The first (+) or (−) represents the $T_i\beta$ hybridization result. The (+) or (−) after the slash (/) represents the presence or absence of the human chromosome in the left column determined by karyotype or isoenzyme analysis (*10, 15*).

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24. Supported by grants GM-09966 (F.H.R.), RO 1 AI 19807 and AI 21226 (E.L.R.), and by a fellowship from the Muscular Dystrophy Association (P.E.B.). We thank A. Hughes for technical assistance and B. Vennstrom for the human c-*erb* B probe λ he-B.

18 June 1984; accepted 2 August 1984